SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

The present study entitled **"Comparison of Various Precipitation Techniques and Partial Purification of Protease isolated from Visceral Organ and Head and Tail Wastes of Indian Oil Sardine (*Sardinella longiceps*) fish"** was aimed at isolating and partially purifying protease from the visceral organ and head and tail wastes of Indian Oil Sardine (*Sardinella longiceps*) *fish* which is one of the most commonly consumed fish in Coimbatore.

The wastes of this fish were collected from a local fish stall in Coimbatore. They were cleaned, homogenized, precipitated with ammonium sulphate, acetone and ethanol in varying concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), purified by dialysis and Sephadex G-100 and the purification profile of the isolated enzyme studied.

The summary of the findings are as follows:

Among the ammonium sulphate precipitated samples of the visceral organ wastes, the 40% precipitated sample registered the highest protein content when compared to all the other samples. Purification by Sephadex G-100 of the ammonium sulphate precipitated sample gave a higher protein content than purification of the ammonium sulphate precipitated sample by dialysis. The study on protease activity showed that precipitation, with 40% ammonium sulphate resulted in the highest protease activity. Precipitation with ammonium sulphate followed by purification by dialysis increased the specific activity of the protease enzyme to the maximum when compared to purification by dialysis. In case of purification fold precipitation with 40% ammonium sulphate followed purification by dialysis recorded the highest value. The maximum

recovery % was with the sample that has been precipitated with 40% ammonium sulphate.

Among the ammonium sulphate precipitated samples of the head and tail wastes of Indian oil sardine (*Sardinella longiceps*), the 50% ammonium sulphate precipitated and Sephadex G-100 run samples registered the highest protein content, when compared to all the other samples. In the case of protease activity, the 50% ammonium sulphate precipitated sample showed the highest value. The activity of the 50% ammonium sulphate also recorded the highest value. In case of purification fold, the precipitation with ammonium sulphate followed by purification with Sephadex G-100 registered the highest purification fold. The study on the recovery % showed that precipitation with 50% ammonium sulphate gave the maximum recovery % of the enzyme protease isolated from the fish wastes.

Purification scheme of the enzyme isolated from the visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) revealed a maximum protein content in 50% acetone precipitated sample.The values of protease activity recorded the highest for 50% acetone precipitated sample. Incase of specific activity the precipitation with 50% acetone registered the highest value. In the case purification fold, that precipitation with acetone followed dialysis increased the purification fold to the maximum extent. The recovery % showed that precipitation with 50% acetone gave the maximum value.

Among the acetone precipitated samples of the head and tail waste, the 60% precipitated sample registered the highest protein content, when compared to all the other samples of the same group. The 60% acetone precipitated sample revealed the highest protease activity. Precipitation with acetone and purification by dialysis of the acetone precipitated samples increased the specific activity of the protease enzyme to the maximum. The precipitation with acetone and purification by dialysis increased the purification fold of the

isolated fish waste enzyme to the maximum when compared to that of the crude. The recovery %was maximum in 60% acetone precipitated sample.

The values of protein content of the ethanol precipitated enzyme sample from visceral organ wastes of Indian oil sardine (Sardinella longiceps) by Sephadex G-100 recorded the maximum protein content for ethanol precipitated + dialysed sample. The study on protease activity showed that precipitation with 50% ethanol increased the protease activity of the fish waste samples to the highest level. Precipitation with 50% ethanol followed by dialysis resulted in the highest specific activity. Purification fold that precipitation with 50% ethanol and purification by dialysis produced the maximum purification fold of the isolated fish waste enzyme compared to that of the crude. It is understood from the findings on recovery % that precipitation with 40% ethanol gave the maximum recovery % of the enzyme isolated from fish wastes.

The values of protein content of the samples isolated from head and tail wastes of Indian oil sardine that precipitation with 40% ethanol recorded the highest value when protease activity also showed similar results. Specific activity also exhibited by the 40 % ethanol precipitated sample. The purification fold showed that precipitation with 40% ethanol followed by purification by dialysis registered the maximum value. The recovery % also showed a maximum value for 40% ethanol precipitated sample.

Hence from the findings of the above study it can be stated that protease enzyme from the visceral organ and head and tail wastes of the selected fish Indian oil sardine (*Sardinella longiceps*) can best be isolated by 40-50% ammonium sulphate 50-60% acetone or 40% ethanol. The isolated enzyme can best be purified by dialysis.

SUGGESTIONS FOR FURTHER RESEARCH

- Various types of proteases can be isolated from fish waste and studied.
- Protease activity from different fish species that exhibit different feeding habits can be analyzed and compared.
- Characterization of protease with respect to activators and inhibitors can be studied.
- The three-dimensional structure of protease can be elucidated and the active sites at which substrate binds can also be found out.
- Protease coding gene of Indian oil sardine (*Sardinella longiceps*) fish can be studied.
- Enzymes other than protease like amylase, cellulase, chitinase, collagenase and phosphatase can be isolated and completely studied from fish waste.